

SUSTAINABLE GREEN FABRICATION OF STARCH/PVA/CUO-NPS ELECTROSPUN NANOSCAFFOLDS FROM *TURBINARIA CONOIDES* (BROWN MARINE MACROALGAE) EXTRACT FOR ENHANCED ANTICANCER ACTIVITY ON HT-29 AND MCF-7 CELLS

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Abstract

This study presents a novel approach for fabricating starch/polyvinyl alcohol (PVA) electrospun nanoscaffolds integrated with biogenically synthesized copper oxide (CuO) nanoparticles derived via ultrasonic-assisted extraction from the brown marine macroalga *Turbinaria conoides*. Comprehensive physicochemical analyses confirmed successful nanoparticle synthesis and uniform incorporation within the polymer matrix, evidenced by UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscopy (SEM), and thermal stability assessments. The CuO nanoparticles exhibited characteristic monoclinic crystal structures and nanoscale dimensions, while the nanoscaffolds demonstrated enhanced thermal stability and homogenous fibrous morphology. Dynamic light scattering (DLS) and zeta potential studies revealed moderate colloidal stability of CuO NPs. Importantly, the fabricated nanocomposites displayed potent, dose-dependent cytotoxicity against HT-29 and MCF-7 cancer cell lines, surpassing the effects of both the raw algal extract and CuO nanoparticles alone. These findings underscore the promising potential of the starch/PVA/CuO nanoscaffolds as effective anticancer agents, warranting further investigation into their therapeutic mechanisms and *in vivo* efficacy for cancer treatment applications.

KEYWORDS: *Turbinaria conoides*, Green synthesis, CuO nanoparticles, Starch/PVA/CuO-NPs nanoscaffolds, Anticancer activity.

1. INTRODUCTION

Nanotechnology has revolutionized the fields of material science and biomedical engineering by enabling the design and fabrication of novel nanoscale materials with enhanced properties and multifunctionality [1]. Among these, metal oxide nanoparticles have gained significant attention for their unique physicochemical characteristics, including high surface area, catalytic activity, and biological effects [2]. In particular, copper oxide nanoparticles (CuO NPs) have emerged as promising candidates in biomedical applications due to their notable antimicrobial, anti-inflammatory, and anticancer properties [3]. However, conventional chemical and physical methods of synthesizing CuO NPs often involve toxic reagents, high energy consumption, and environmental hazards, limiting their biomedical applicability [3]. This challenge has led to increased interest in developing green synthesis routes that employ biological resources for the eco-friendly production of nanoparticles [4].

Biogenic synthesis of nanoparticles using plant and algal extracts offers a sustainable and cost-effective alternative by harnessing the inherent reducing, capping, and stabilizing agents present in natural biomolecules such as polyphenols, flavonoids, proteins, and polysaccharides [5]. Marine macroalgae, commonly known as seaweeds, represent a prolific reservoir of bioactive compounds with diverse chemical structures and biological functions [6]. Among these, *Turbinaria conoides*, a brown marine macroalga abundantly found along the coastal regions of India, has demonstrated a rich profile of polysaccharides, phenolic compounds, and antioxidants [7]. These biomolecules not only facilitate the rapid reduction of metal ions but also enhance the stability and functionality of the synthesized nanoparticles [8]. Despite this potential, few studies have explored the ultrasonic-assisted extraction of *T. conoides* phytoconstituents specifically for the green synthesis of CuO NPs, which could improve extraction efficiency and nanoparticle quality by promoting better cell wall disruption and metabolite release [9].

In parallel, the development of nanofibrous scaffolds for biomedical applications, particularly in tissue engineering and drug delivery, has witnessed remarkable advancements through electrospinning technology [10]. Electrospinning enables the fabrication of continuous fibers with diameters ranging from the nanometer to micrometer scale, closely mimicking the extracellular matrix (ECM) architecture [11]. Such scaffolds provide a highly porous and biocompatible environment conducive to cell attachment, proliferation, and differentiation [12]. Polyvinyl alcohol (PVA), a synthetic hydrophilic polymer, has been widely used in electrospinning due to its excellent film-forming ability, biocompatibility, and mechanical strength [13]. However, PVA alone lacks bioactivity and rapid biodegradability. To overcome these limitations, natural polymers like starch are incorporated to improve biodegradability, enhance biological interactions, and reduce the reliance on synthetic polymers [14]. Starch, a renewable polysaccharide, is especially attractive owing to its abundance, low cost, and inherent biocompatibility, making it an ideal candidate to form composite scaffolds with PVA [15].

Integrating biogenic metal oxide nanoparticles within polymeric nanofibrous matrices offers a promising strategy to develop multifunctional scaffolds that combine mechanical integrity with therapeutic activity [16]. Embedding CuO NPs into starch/PVA electrospun scaffolds can potentially impart anticancer and antimicrobial functionalities, while maintaining favorable physicochemical properties essential for biomedical use [17]. However, achieving uniform nanoparticle dispersion and stable incorporation without compromising fiber morphology or scaffold performance remains a critical challenge [16]. Employing ultrasonic-assisted green synthesis of CuO NPs from *T. conoides* extract and their subsequent incorporation into starch/PVA nanofibers represents an innovative approach to address these issues [17]. This methodology leverages the synergistic advantages of eco-friendly nanoparticle synthesis, efficient phytochemical extraction, and advanced electrospinning fabrication to yield functional nanoscaffolds with potential anticancer efficacy [18].

Cancer remains a leading cause of mortality worldwide, and conventional chemotherapy often suffers from severe side effects and drug resistance [19]. Nanomaterial-based therapies offer targeted and controlled delivery options that could enhance therapeutic indices and minimize adverse reactions [20]. CuO NPs have been reported to induce reactive oxygen species (ROS) generation selectively in cancer cells, triggering apoptosis and inhibiting tumor progression [21]. By embedding these nanoparticles within biodegradable nanoscaffolds, a localized and sustained anticancer effect can be achieved, which may improve treatment outcomes and reduce systemic toxicity [22]. Despite these promising prospects, comprehensive studies on the physicochemical characterization, biological compatibility, and anticancer activity of such biogenic CuO NP-incorporated starch/PVA scaffolds are scarce, especially when the nanoparticles are synthesized using marine macroalgal extracts [23].

Therefore, this study aims to develop a novel starch/PVA electrospun nanofibrous scaffold embedded with CuO NPs synthesized via an ultrasonic-assisted green method using *T. conoides* extract. The objectives include the optimization of phytochemical extraction from *T. conoides* by ultrasonication, the eco-friendly biosynthesis of CuO NPs [9], and the fabrication of uniform composite nanoscaffolds through electrospinning [24]. Extensive physicochemical characterization techniques such as UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscopy (SEM), Thermogravimetric analysis (TGA), and particle size analysis are employed to elucidate nanoparticle formation, structural integration, fiber morphology, thermal stability, and colloidal properties [25]. Moreover, the anticancer potential of the synthesized scaffolds is evaluated against human colon (HT-29) and breast (MCF-7) cancer cell lines using standard cytotoxicity assays [26].

The innovative combination of marine macroalgal biogenic synthesis and starch/PVA electrospun scaffolds presented in this work offers an environmentally sustainable and potentially effective platform for anticancer applications. The integration of bioactive CuO NPs derived from *T. conoides* extract within a biocompatible polymer matrix addresses current challenges in nanomedicine related to toxicity, stability, and multifunctionality. This research could pave the way for developing next-generation nanomaterials for cancer therapeutics and tissue engineering, contributing to the broader goals of green nanotechnology and precision medicine.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

For this study, *T. conoides*, a brown seaweed native to the coastal region of Rameswaram, Tamil Nadu, India, was selected for the green synthesis of CuO NPs. Copper (II) sulfate pentahydrate, of analytical purity, served as the metal precursor. PVA, having an approximate molecular weight of 115,000 g/mol, and starch were used to produce nanofibrous scaffolds via electrospinning using a HOLMARC HO-NFES-040 system. All required solvents and chemical agents, sourced from Merck and Sigma-Aldrich, were of analytical grade to ensure experimental reproducibility. The synthesized materials underwent detailed physicochemical characterization and were further assessed for their anticancer efficacy through established bioassays.

2.2. Collection and preparation of algal biomass

Fresh specimens of *T. conoides* were collected manually from the intertidal zones along the Rameswaram coast. The samples were immediately transferred to the laboratory in ice-filled containers. Surface impurities such as epiphytes and sediments were removed through thorough rinsing with tap water, followed by multiple washes using distilled water to eliminate salt residues. Approximately 100 g of cleaned algal thalli were chopped into

small sections and air-dried at room temperature for 14 days. Once completely dried, the algal material was finely ground and stored in airtight containers for further extraction and nanoparticle synthesis.

2.3. Ultrasonic extraction of phytoconstituents

To extract phytochemicals, 2 g of the powdered algal biomass was added to 50 mL of Milli-Q water in a 100 mL borosilicate container. The suspension underwent ultrasonic-assisted extraction using a LABQUEST ultrasonic cleaner (Borosil, Serial No. 941032329018) at 60°C for 45 minutes to enhance cellular disruption and metabolite release. The extract was filtered through Whatman No. 1 filter paper to remove particulate matter. The clear extract was refrigerated at 10°C until further use, and a portion was freeze-dried to obtain a stable powdered extract suitable for nanoparticle synthesis [27, 28].

2.4. Eco-friendly synthesis of CuO NPs

For biosynthesis, 50 mL of 0.1 M copper sulfate solution was combined with 10 mL of algal extract (10 mg dry weight equivalent) at a 5:1 ratio. This solution was continuously stirred at 650 rpm and maintained at 60°C for 2 hours using a magnetic stirrer with temperature control. A visible color change from deep blue to bluish-green indicated successful reduction of copper ions by the algal phytochemicals. The resultant nanoparticles were washed three times with double-distilled water to remove unreacted components, then freeze-dried for 48 hours to obtain a powdered form of CuO NPs [29].

2.5. Fabrication of starch/PVA/CuO NP nanofiber scaffolds

To fabricate nanoscaffolds, a 15% (w/w) aqueous PVA solution was prepared by dissolving the polymer in Milli-Q water with constant stirring at 50°C. After complete solubilization, 100 mg of starch and synthesized CuO NPs were incorporated into the solution. This mixture was stirred for an additional 2.5 hours at the same temperature to ensure homogenous dispersion. The final blend was transferred into a syringe with a 0.84 mm stainless steel needle for electrospinning on a HOLMARC HO-NFES-040 unit. The setup operated at room temperature with optimized parameters: 11.5 kV voltage, 12.3 cm needle-to-collector distance, and 0.4 mL/h flow rate. Electrospinning was carried out for 6 to 8 hours to form uniform nanofibers, which were collected and stored in desiccators for later characterization [30-32].

2.6. Physicochemical characterization

2.6.1. UV-visible spectroscopy

UV-Vis spectral analysis was performed using a VIS SPEC V-730 spectrophotometer to assess the optical features of algal extract, CuO NPs, and composite nanoscaffolds. Absorbance was recorded over the 200 to 800 nm range using deionized water as a blank. Specific absorption bands between 270 and 300 nm confirmed nanoparticle synthesis and scaffold incorporation [33].

2.6.2. FTIR spectroscopy analysis

FTIR spectroscopy was conducted using a PerkinElmer Spectrum Two in ATR mode to analyze functional groups and molecular interactions. Each sample was scanned in the 4000–400 cm^{-1} range with 64 scans and a 4 cm^{-1} resolution. Peaks attributed to hydroxyl, carbonyl, amide, and metal-oxygen bonds verified both phytochemical presence and nanoparticle inclusion [34].

2.6.3. XRD analysis

Crystalline structure and phase analysis of CuO NPs and their integration within scaffolds were assessed using a Bruker D8 Advance diffractometer with $\text{CuK}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$). Scans ranged from 5° to 90° 2 θ at 40 kV and 40 mA, with a 0.029649° step size. Distinct peaks representing monoclinic CuO confirmed crystallinity; broadened peaks in the nanocomposites indicated good nanoparticle dispersion [35].

2.6.4. SEM analysis

Surface morphology and fiber uniformity were examined via a JEOL JSM-IT800 NANO SEM. Samples were coated with a thin layer of gold to reduce charging. The SEM images demonstrated even fiber diameters and uniform CuO NP distribution, affirming successful electrospinning [36].

2.6.5. TGA

TGA was used to assess the thermal properties using a PerkinElmer TGA 8000. About 5 mg of each sample was heated from room temperature to 550°C at 15°C/min in a nitrogen atmosphere. The thermograms illustrated multiple degradation stages, highlighting moisture loss, polymer decomposition, and enhanced thermal stability due to nanoparticle reinforcement [37].

2.6.6. Particle size and zeta potential measurements

Size and surface charge of CuO NPs were determined using a Malvern Zetasizer Ultra. Hydrodynamic diameter was measured using Dynamic Light Scattering (DLS), while zeta potential assessed colloidal stability. Nanoparticles with zeta potential values beyond $\pm 30 \text{ mV}$ were deemed stable for biological applications [38].

Characterization data are publicly available on Mendeley Data (DOI: 10.17632/mmyv8mmkxbx.2).

2.7. In vitro anticancer activity

2.7.1. Cell lines (HT-29 and MCF-7) procurement and culture

Human colon cancer (HT-29) and breast cancer (MCF-7) cell lines were obtained from the National Center for Cell Science (NCCS), Pune, India. Cells were cultured following standard protocols in Minimum Essential Medium (MEM) with 10% fetal bovine serum (FBS) at 37°C under 5% CO_2 in a humidified incubator [26].

2.7.2. MTT assay for cell viability

Cells were seeded in 96-well plates at 5×10^5 cells/well and allowed to adhere overnight. They were then exposed to varying concentrations (6.25, 12.5, 25, 50, 100 $\mu\text{g/mL}$) of algal extract, CuO NPs, and starch/PVA/CuO-NP nanoscaffolds for 24 hours. Following incubation, MTT reagent was added and the cells were incubated for an additional 4 hours. Formazan crystals formed were solubilized with 200 μL DMSO, and absorbance was measured at 570 nm using a microplate reader. Experiments were repeated thrice independently for accuracy. The mean optical density and standard deviation were calculated to interpret cytotoxicity effects [39].

2.8. Statistical analysis

Statistical evaluations were performed using SPSS v25.0 and GraphPad Prism v9.0. Data from triplicate experiments were expressed as mean $\pm$ standard deviation. One-way ANOVA with appropriate post hoc tests (Tukey's or Dunnett's) determined group differences, with significance set at $p < 0.05$. Pearson's correlation and Shapiro–Wilk normality tests were also applied. If needed, data were log-transformed to meet normality criteria. All results were analyzed within a 95% confidence level to ensure statistical rigor [40].

3. RESULTS AND DISCUSSION

3.1. UV-visible spectroscopy

The UV–Visible absorption spectra of *T. conoides* aqueous extract, the biosynthesized CuO NPs, and the CuO-loaded starch/PVA nanoscaffolds were recorded using a JASCO V-730 spectrophotometer within a wavelength range of 200–800 nm (Fig. 1). The crude seaweed extract exhibited broad absorption peaks in the lower wavelength region, which is typical for compounds such as flavonoids and polyphenols that absorb ultraviolet light due to their conjugated π -electron structures.

The biosynthesized CuO NPs showed a distinct, sharp absorption peak in the 270–300 nm range, indicative of surface plasmon resonance (SPR). This SPR phenomenon arises from the collective oscillation of conduction electrons on the nanoparticle surface excited by light. The clear SPR peak confirms the successful biosynthesis of CuO NPs from Cu^{2+} ions, mediated by the seaweed's bioactive compounds.

When embedded within starch/PVA nanofibers, the CuO NPs caused subtle spectral shifts and broadening of absorption peaks relative to pure CuO NPs. These spectral changes imply interactions at the molecular level, likely hydrogen bonding or van der Waals forces, between CuO NPs and the polymer chains. This confirms the effective encapsulation and uniform dispersion of CuO NPs throughout the scaffold. All measurements were performed in triplicate for reliability, using deionized water as the baseline reference to remove background noise [41–43].

3.2. FTIR spectroscopy analysis

FTIR spectroscopy was employed to elucidate the functional groups involved in nanoparticle stabilization and their interaction with the polymer matrix. The spectra of CuO NPs and CuO-loaded scaffolds were recorded via a PerkinElmer Spectrum Two FTIR spectrometer in ATR mode, scanning between 4000 and 400 cm^{-1} at 4 cm^{-1} resolution over 64 scans.

The FTIR spectrum of the CuO NPs (Fig. 2) revealed characteristic Cu–O stretching vibrations at 590.53 cm^{-1} and 464.53 cm^{-1} , confirming copper oxide formation. Additional peaks at 801.66 cm^{-1} corresponded to C–H bending, while 1015.22 cm^{-1} and 1065.56 cm^{-1} were assigned to C–O stretching modes. The absorption at 1511.28 cm^{-1} represented aromatic C=C or N–H bending vibrations. A broad O–H stretching band appeared at 3166.88 cm^{-1} , indicating the presence of phytochemicals from the algae adsorbed onto the nanoparticle surface. In the CuO-loaded starch/PVA scaffold, the FTIR spectra showed new and shifted peaks confirming nanoparticle incorporation. The Cu–O band appeared at 603.51 cm^{-1} , reaffirming CuO presence. Other prominent bands included 843.42 cm^{-1} (C–H bending), 913.12 cm^{-1} (C–O–C stretching), and 1089.99 cm^{-1} (C–O stretching), characteristic of polysaccharide and ether groups. Strong vibrations at 1425.37 cm^{-1} and 2923.53 cm^{-1} were linked to C–H groups, while peaks at 1711.84 cm^{-1} and 1732.70 cm^{-1} indicated ester and carbonyl groups from PVA. The broad O–H stretch at 3296.86 cm^{-1} suggested hydrogen bonding, supporting a stable interaction network between polymer and nanoparticles [44, 45].

3.3. XRD analysis

XRD was performed to assess the crystalline structure and the integration of CuO NPs within the nanoscaffolds. A Bruker D8 Advance diffractometer with $\text{CuK}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$) was used at 40 kV and 40 mA, scanning 2θ from 5° to 90° with a step size of 0.029649° (Fig. 3).

The diffraction pattern of the CuO NPs displayed sharp, well-defined peaks characteristic of a monoclinic crystal phase, consistent with JCPDS card No. 48-1548. Notable reflections were observed at 15.666° , 16.451° , 17.368° , 20.181° , 24.173° , 26.071° , 28.492° , 31.764° , 32.614° , 35.035° , 38.242° , and 47.468° . The most intense peak at 26.071° corresponded to a d-spacing of $3.41519 \text{ \AA}$ and a narrow full width at half maximum (FWHM) of 0.100° , suggesting small crystallite sizes.

The XRD pattern of the CuO-loaded starch/PVA scaffold showed semi-crystalline characteristics. Peaks at 11.332° , 19.284° , 21.272° , 23.481° , 29.372° , 30.771° , 35.778° , 39.533° , and 47.559° were recorded. The strong reflection at 29.372° ($d = 3.03842 \text{ \AA}$, FWHM = 0.170°) indicated contributions from the polymer matrix. CuO-specific peaks at 30.771° and 35.778° confirmed nanoparticle retention within the scaffold. Peak broadening and shifts suggest reduced crystallite size and internal strain caused by polymer–nanoparticle interactions. The monoclinic phase of CuO remained unchanged, demonstrating that its crystal structure was preserved after embedding [38, 46].

3.4. SEM analysis

Surface morphology of CuO NPs and nanoscaffolds was examined using a JEOL JSM-IT800 NANO scanning electron microscope. Prior to imaging, samples were coated with gold to enhance electrical conductivity.

SEM micrographs of CuO NPs revealed predominantly spherical particles with occasional irregular shapes, sized between 60 and 90 nm. Some degree of aggregation was observed, likely due to the high surface energy of nanoparticles causing mutual attraction. SEM images of CuO-starch/PVA scaffolds (Fig. 4) displayed uniform fibrous networks with fiber diameters ranging from 90 to 110 nm. The fibers exhibited smooth surfaces and interconnected porosity, advantageous for applications such as drug delivery and tissue engineering by promoting cell adhesion and nutrient transport.

CuO NPs appeared well embedded within the fibers, distributed evenly with minimal aggregation, indicating effective incorporation through electrospinning. The consistent nanoparticle dispersion and uniform fiber formation demonstrate optimized processing parameters for fabricating stable, functional nanocomposite scaffolds [47, 48].

3.5. TGA

Thermal stability of CuO-loaded nanofibers was analyzed with a PerkinElmer TGA 8000 instrument. A 7.685 mg sample was heated from 30°C to 550°C at 15°C/min under nitrogen atmosphere (Fig. 5). The thermogram revealed two distinct degradation phases. The first started at 326.78°C and peaked at 381.60°C, corresponding to a mass loss of 34.960%, associated with decomposition of starch, PVA, and volatile organics. The second phase commenced at 438.61°C, reaching a maximum at 459.06°C, with an additional 4.679% weight loss attributed to degradation of residual organic materials and bioactives bound to CuO. Compared to pure starch/PVA scaffolds, the CuO-containing composites exhibited delayed thermal degradation onset, demonstrating enhanced thermal stability. This effect is attributed to the inorganic CuO restricting polymer chain mobility and acting as a thermal barrier. The derivative thermogravimetry (DTG) further clarified these transitions. Minimal residual mass at 550°C indicated nearly complete organic decomposition, with CuO functioning as a thermal stabilizer within the matrix [49-51].

3.6. Zeta potential and particle size analysis

DLS and zeta potential measurements were carried out using a Malvern Zetasizer Ultra to determine particle size distribution and surface charge of CuO NPs. The hydrodynamic diameter distribution showed a predominant peak at 68.12 nm (Fig. 6), consistent with SEM size observations. The polydispersity index (PDI) was 0.5717, indicating moderate heterogeneity in particle sizes.

Zeta potential analysis revealed a primary surface charge of -11.39 mV with minor peaks at -42.6 mV and -12.28 mV (Fig. 7). The main zeta value suggests limited electrostatic repulsion between particles, allowing some aggregation potential over time. However, the presence of a more negatively charged fraction implies partial colloidal stability. Overall, the system's zeta potential is below the ± 30 mV threshold generally regarded as indicative of strong stability. Conductivity was measured at 0.06892 mS/cm, and the count rate was 3.27×10^4 kcps, supporting reasonable dispersion quality. For enhanced long-term colloidal stability in biomedical contexts, further surface modification or stabilizing agents may be necessary. These results provide critical insights into the stability and applicability of CuO NPs for biomedical use [52, 53].

3.7. Anticancer activity analysis

The *in vitro* anticancer activity of the algal extract, CuO NPs, and starch/PVA/CuO-NPs nanoscaffolds was evaluated against HT-29 (human colon adenocarcinoma) and MCF-7 (breast cancer) cell lines using the MTT assay. Cell viability was measured at concentrations ranging from 6.25 to 100 $\mu\text{g/mL}$, with untreated cells (0 $\mu\text{g/mL}$) serving as the control (100% viability).

Against HT-29 cells, the algal extract exhibited moderate cytotoxicity, reducing cell viability from 93.5% at 6.25 $\mu\text{g/mL}$ to 32.25% at 100 $\mu\text{g/mL}$, with an estimated IC₅₀ value of approximately 35 $\mu\text{g/mL}$. CuO NPs demonstrated stronger activity, decreasing viability from 78.5% to 21.83% over the same concentration range, yielding an IC₅₀ of about 18 $\mu\text{g/mL}$. The starch/PVA/CuO-NPs nanoscaffolds were the most effective, showing a sharp decline in viability from 74.9% at 6.25 $\mu\text{g/mL}$ to 14.88% at 100 $\mu\text{g/mL}$, with an IC₅₀ of approximately 12 $\mu\text{g/mL}$.

Similar trends were observed in MCF-7 cells. The algal extract reduced viability from 95.3% to 46.05%, with an IC₅₀ of around 40 $\mu\text{g/mL}$. CuO NPs exhibited greater potency, lowering viability from 84.8% to 37.47% and achieving an IC₅₀ of about 22 $\mu\text{g/mL}$. Once again, the nanoscaffolds displayed the highest efficacy, reducing viability from 78.4% to 24.42% and producing an IC₅₀ of roughly 15 $\mu\text{g/mL}$.

These findings highlight the dose-dependent anticancer activity of all tested compounds, with the starch/PVA/CuO-NPs nanoscaffolds emerging as the most potent. Their superior cytotoxicity compared to CuO NPs and the algal extract suggests potential for further development as an anticancer therapeutic. Future studies should explore their mechanisms of action and *in vivo* performance to validate these promising results [54, 55].

5. CONCLUSION

This study successfully demonstrated the green synthesis of CuO NPs using *T. conoides* extract through an ultrasonic-assisted method and their effective incorporation into starch/PVA electrospun nanoscaffolds.

Physicochemical characterizations confirmed the preservation of CuO's monoclinic crystalline structure and strong interactions between nanoparticles and polymer matrices, contributing to improved thermal stability and uniform fibrous morphology. The embedded CuO NPs exhibited well-dispersed nanoscale dimensions, facilitating enhanced bioavailability within the scaffold framework. Importantly, the developed nanocomposites exhibited superior anticancer activity against HT-29 and MCF-7 cell lines compared to individual components, indicating synergistic effects from nanoparticle-polymer integration. These findings highlight the potential of these biogenic CuO-loaded starch/PVA nanoscaffolds as promising candidates for anticancer therapeutics. Future research should focus on elucidating the molecular mechanisms underlying their cytotoxic effects and evaluating their performance in *in vivo* cancer models to advance clinical applicability. Overall, this study paves the way for eco-friendly, effective nanomaterial-based cancer treatments.

Statements and Declarations

Ethics declaration: Not Applicable

Funding Declaration: This study was funded by Lucian Blaga University of Sibiu & Hasso Plattner Foundation research grants LBUS-IRG-2022-08.

Clinical trial number: Not applicable

Consent to Publish declaration: Not applicable

Consent to Participate declaration: Not applicable

Author Contribution declaration: **JinJin Pei and Gopika Mohanakumar:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft and Funding acquisition. **Kamel Hessini, Suresh Subramaniam, Selvaraj Jayaraman, Sivaramakrishnan R, Monica Mironescu and Gopalakrishnan Velliur Kanniappan:** Conceptualization, Methodology, Resources and Formal analysis. **Ion Dan Mironescu and Chella Perumal Palanisamy:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration and Funding acquisition

Conflicts of Interest: The authors declare no conflicts of interest.

Data Availability Statement: The full dataset of physiochemical characterization associated with this work is published in Mendeley Data (doi: 10.17632/mmyv8mmkxb.2). **Disclosure:** The material characterization data and figures (e.g., UV, SEM, FTIR, XRD, TGA, Zeta Potential and particle sizer) presented here were generated from a common dataset and are being reused across multiple related studies on nanomaterials. All biological and environmental analyses and conclusions are original to each manuscript.

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Figures

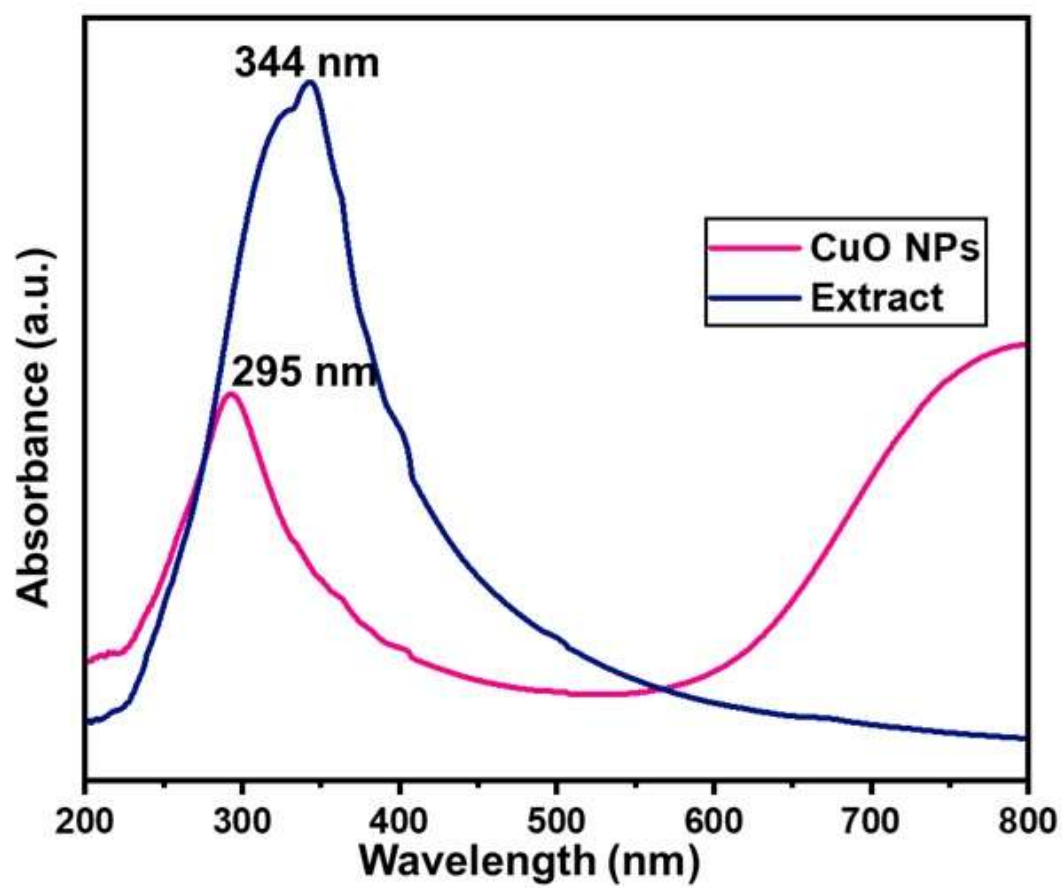


Fig. 1. UV–Visible spectral profiles of algal extract and CuO NPs.

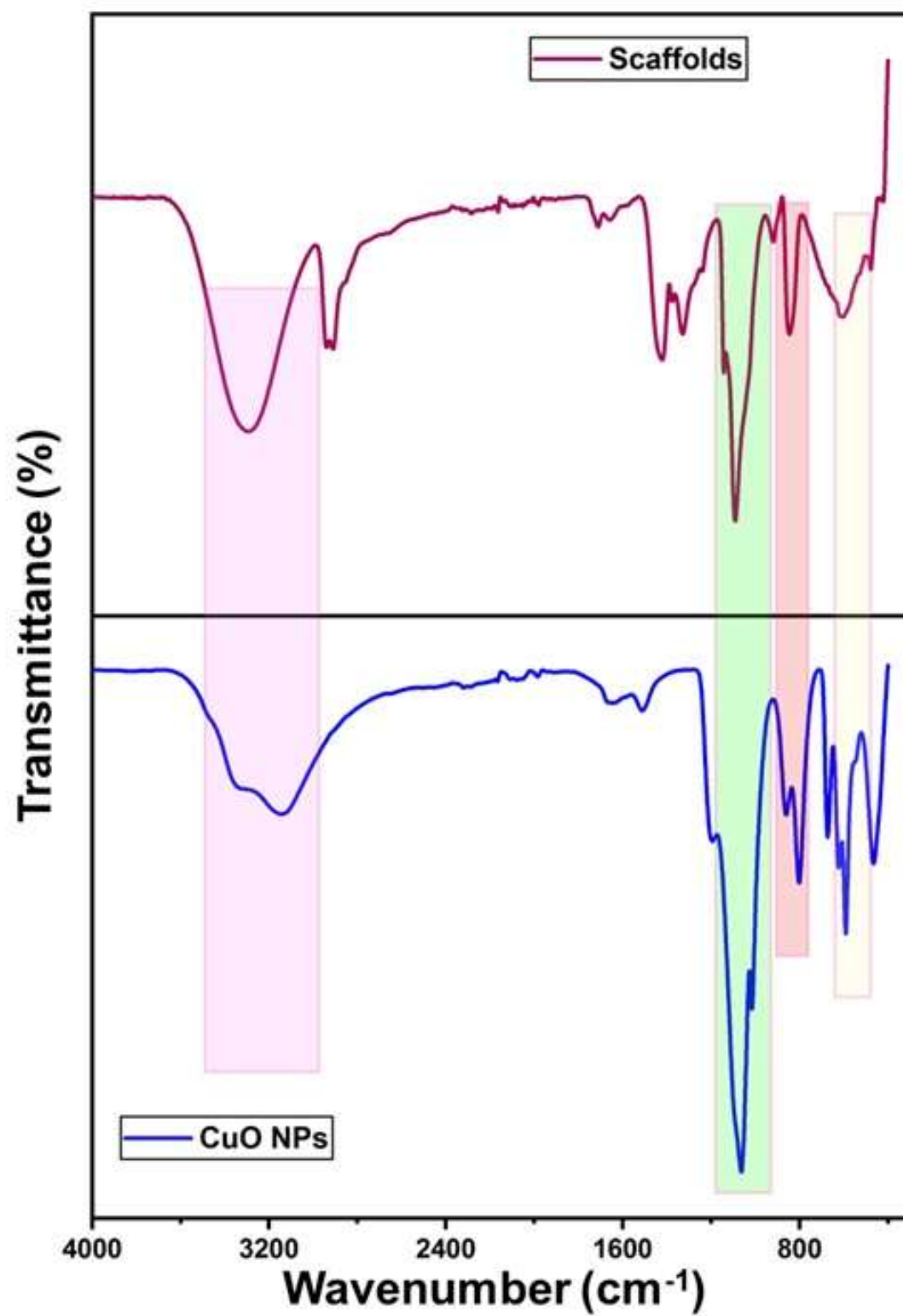


Fig. 2. FTIR spectra of CuO NPs and their incorporation into nanoscaffolds.

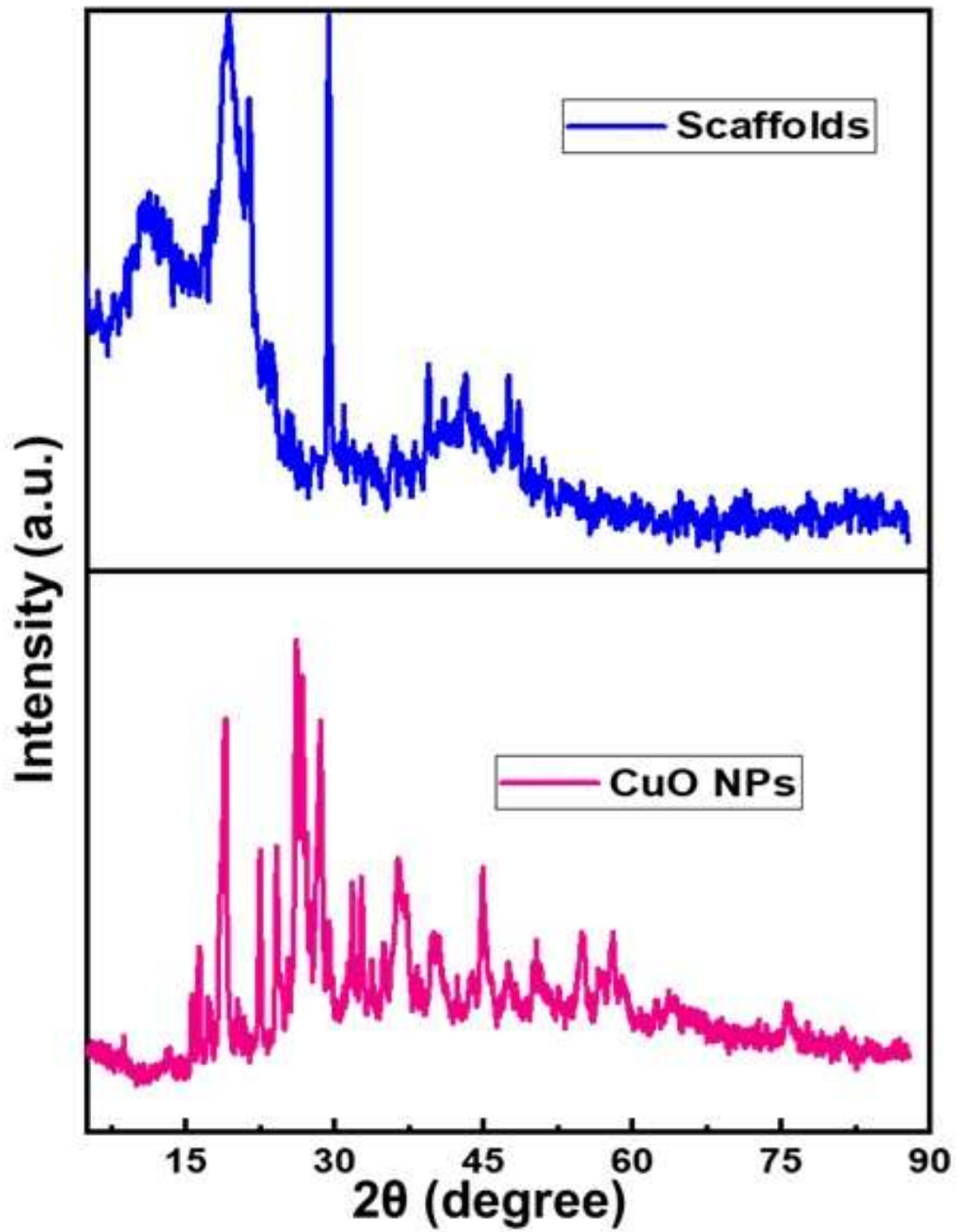


Fig. 3. XRD patterns of synthesized CuO NPs and CuO-loaded scaffolds.

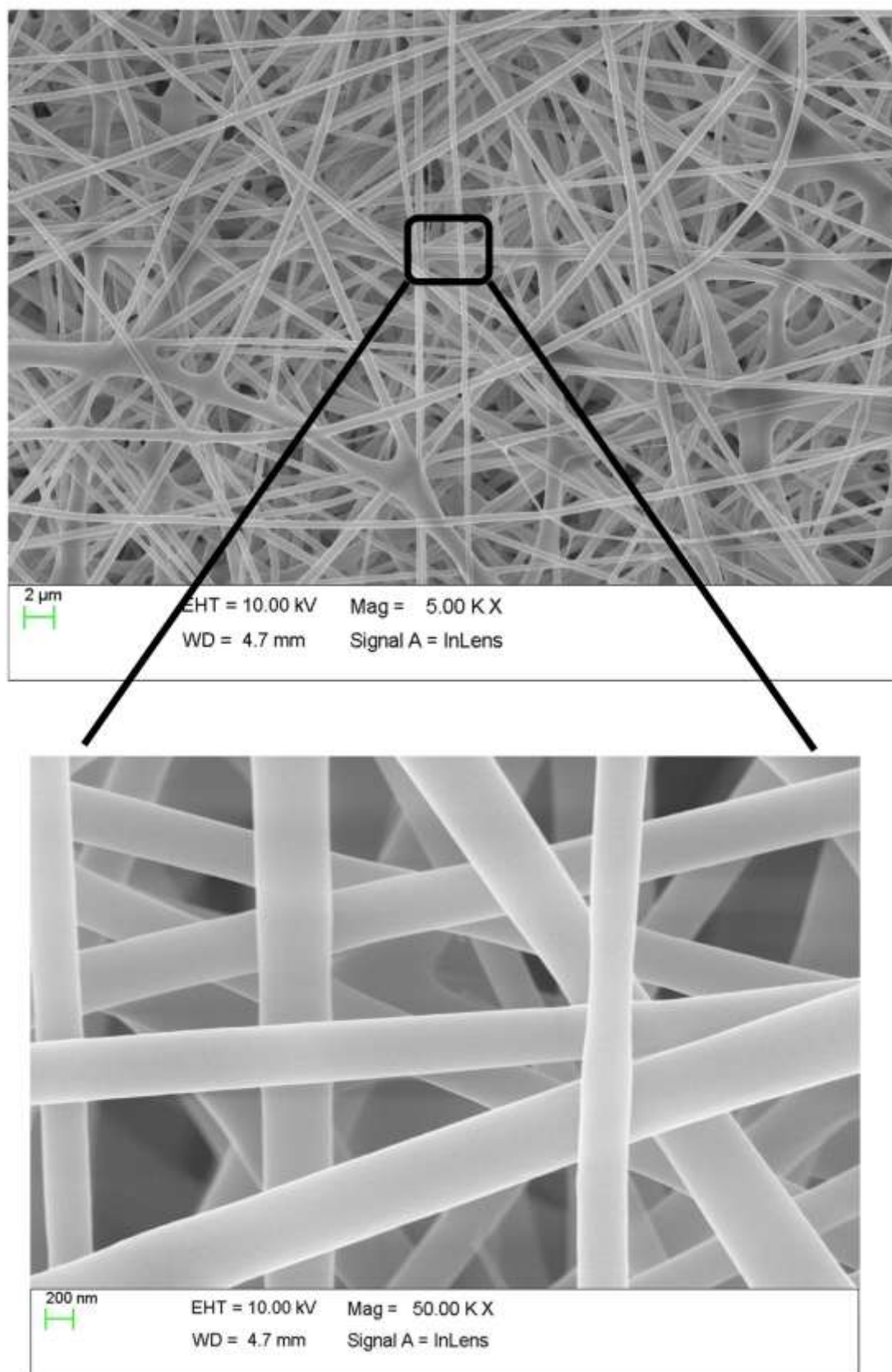


Fig.4. SEM analysis of starch/PVA/CuO-NPs nanoscaffolds

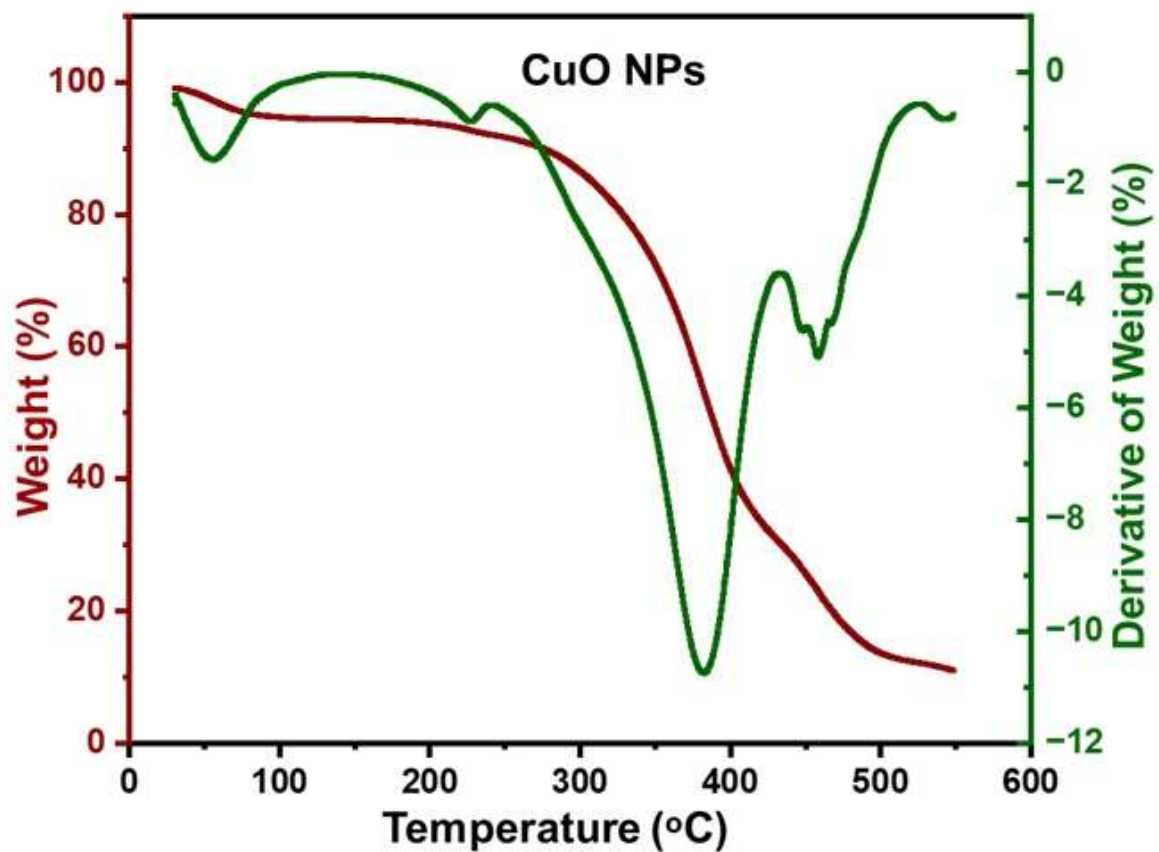


Fig. 5. Thermal degradation behavior of CuO-incorporated nanoscaffolds.

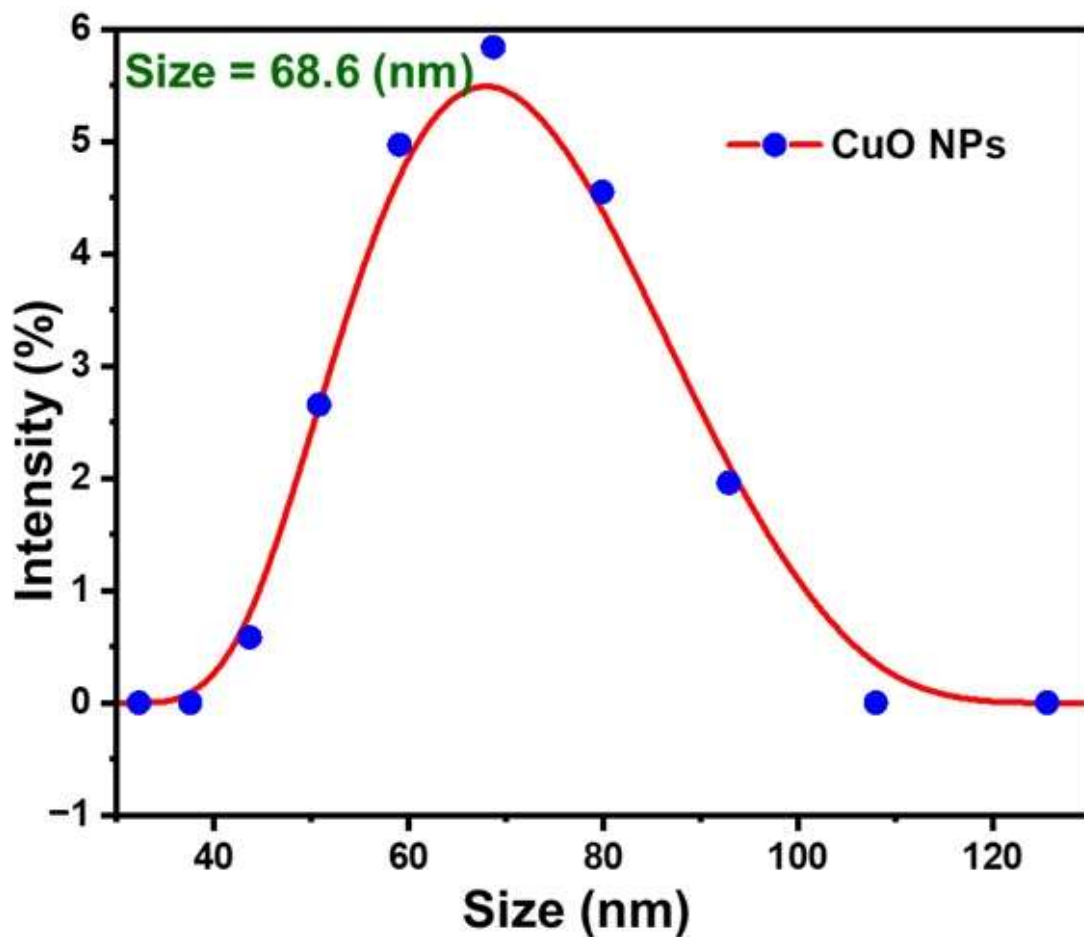


Fig.6. DLS particles size analysis of CuO NPs

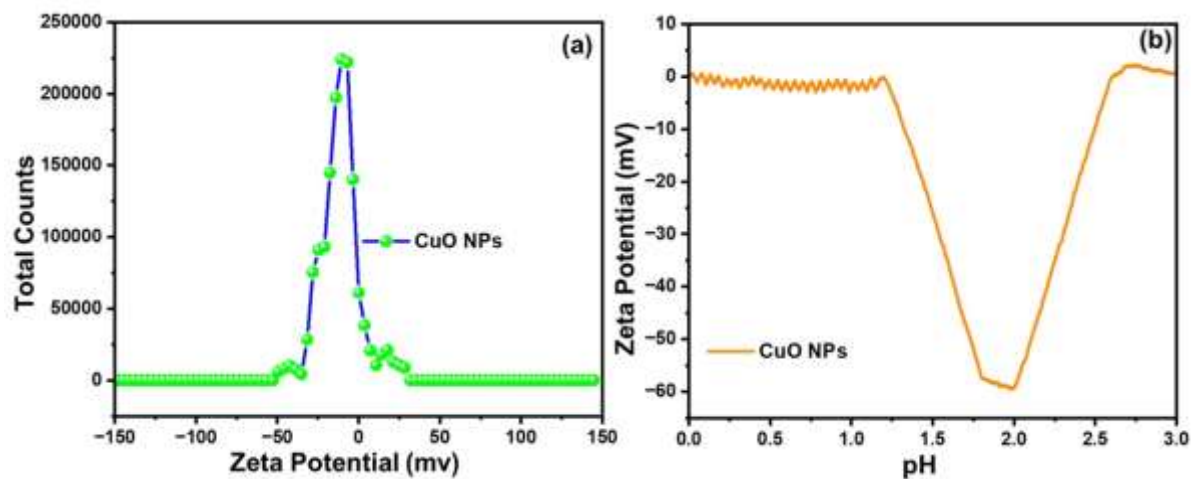


Fig.7. Zeta-potential analysis of CuO NPs

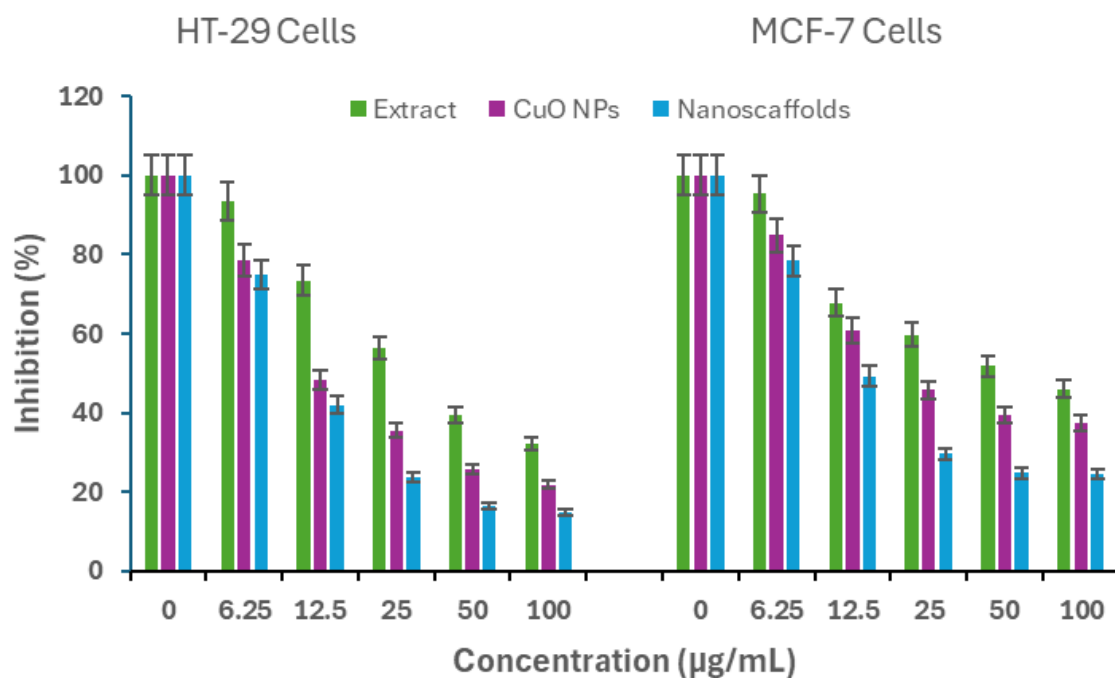


Fig.8. Anticancer activity analysis of algal extract, CuNPs and starch/PVA/CuONPs nanoscaffolds